

CLAIMS

1. A process for producing a fermentation product from milled starch-containing material comprising:
 - (a) saccharifying milled starch-containing material with a glucoamylase having an amino acid sequence shown in SEQ ID NO: 2, or a glucoamylase being at least 70% identical thereto, at a temperature below the initial gelatinization temperature of said starch-containing material,
 - (b) fermenting using a fermenting organism.
2. The process of claim 1, wherein the process is carried out for a period of 1 to 250 hours, preferably is from 25 to 190 hours, more preferably from 30 to 180 hours, more preferably from 40 to 170 hours, even more preferably from 50 to 160 hours, yet more preferably from 60 to 150 hours, even yet more preferably from 70 to 140 hours, and most preferably from 80 to 130 hours.
3. The process of claim 1 or 2, wherein the process is carried out at a pH in the range between 3 and 7, preferably from 3.5 to 6, or more preferably from 4-5.
4. The process of any of claims 1 to 3, wherein the dry solid content (DS) lies in the range from 20-55 wt.-%, preferably 25-40 wt.-%, more preferably 30-35 wt.-%.
5. The process of any of claims 1-4, wherein the sugar concentration is kept at a level below about 3 wt. % during saccharification and fermentation.
6. The process of any of claims 1-5, wherein a slurry comprising water and milled starch-containing material is prepared before step (a).
7. The process of any of claims 1-6, wherein the milled starch-containing material is prepared by milling starch-containing material to a particle size of 0.1-0.5 mm.
8. The process of any of claims 1-7 wherein the milled starch-containing material is granular starch obtained by dry or wet milling.
9. The process of any of claims 1-8, wherein the milled starch containing material is whole grains.

10. The process of any of claims 1-9, wherein the saccharification is carried out simultaneously.

11. The process of claim 10, wherein the temperature during fermentation is between 28°C and 36°C, such as between 29°C and 35°C, such as between 30°C and 34°C, such as around 32°C.

12. The process of any of claims 1-3, wherein the glucoamylase is derived from *Athelia rolfsii*.

13. The process of any of claims 1-13, wherein the glucoamylase is present in an amount of 0.001 to 10 AGU/g DS, preferably from 0.01 to 5 AGU/g DS, especially 0.1 to 0.5 AGU/g DS.

14. The process of any of claims 1-13, wherein an acid alpha-amylase is present.

15. The process of claim 14, wherein the acid alpha-amylase is a fungal alpha-amylase, preferably derived from a strain of *Aspergillus*, especially *A. niger*, *A. oryzae*, or *A. awamori*.

16. The process of claim 15, wherein the acid alpha-amylase is an acid alpha-amylase having an amino acid sequence which has at least 70%, preferably at least 75%, 80%, 85% or at least 90%, e.g. at least 95%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4.

17. The process of any of claims 14-16, wherein the fungal acid alpha-amylase is a hybrid enzyme comprising an alpha-amylase catalytic domain (CD) and a carbohydrate-binding module (CBM) and optionally linker or a wild-type fungal acid alpha-amylase catalytic domain (CD) and a carbohydrate-binding module (CBM) and optionally a linker.

18. The process of claim 17, wherein the CBM is derived from *Aspergillus kawachii* alpha-amylase, *Athelia rolfsii* glucoamylase, or *Aspergillus niger* glucoamylase.

19. The process of claim 17 or 18, wherein the CBM is derived from *Athelia rolfsii* glucoamylase, *A. niger* glucoamylase or *A. kawachii* alpha-amylase.

20. The process of claim 14, wherein the acid alpha-amylase is an acid bacterial alpha-amylase.

21. The process of claim 20, wherein the acid alpha-amylase is derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*.

22. The process of claim 21, wherein the acid bacterial alpha-amylase is derived from a strain of *Bacillus stearothermophilus*, having the mutations I181* + G182*, preferably I181* + G182*, + N193F compared to the wild type amino acid sequence set forth in SEQ ID NO: 7.

23. The process of claim 20, wherein the bacterial alpha-amylase is a hybrid alpha-amylase comprising the 445 C-terminal amino acid residues of the *Bacillus licheniformis* alpha-amylase set forth in SEQ ID NO: 5 and the 37 N-terminal amino acid residues of the alpha-amylase derived from *Bacillus amyloliquefaciens* set forth in SEQ ID NO: 6 having the substitution G48A + T49I + G107A + H156Y + A181T + N190F + I201F + A209V + Q264S (using the SEQ ID NO: 5 numbered).

24. The process of claim 20, wherein the bacterial alpha-amylase is an alpha-amylase having the amino acid sequence set forth in SEQ ID NO: 5 having the mutations H156Y, A181T, N190F, A209V, Q264S, and/or deletion of two residues between positions 176 and 179, preferably deletion of E178 and G179.

25. The process of any of claims 14-24, wherein the acid alpha-amylase is present in an amount of 0.1 to 10 AFAU/g DS, preferably 0.10 to 5 AFAU/g DS, especially 0.3 to 2 AFAU/g DS.

26. The process according to any of claims 14-25, wherein the acid alpha-amylase and glucoamylase is added in a ratio of between 0.1 to 10 AGU/AFAU, preferably 0.30 and 5 AFAU/AGU, especially 0.5 to 3 AFAU/AGU.

27. The process of any of claims 1-26, wherein the fermentation product is recovered after fermentation.

28. The process of any of claims 1-27, wherein the fermentation product is an alcohol, preferably ethanol, especially fuel ethanol, potable ethanol and/or industrial ethanol.

29. The process of any of claims 1-28, wherein the process is performed in the presence of a protease, preferably an acid protease, preferably a fungal acid protease.

30. The process of any of claims 1-29, wherein the starch-containing material is obtained from tubers, roots, stems, fruits, seeds or whole grain.

31. The process of any of claims 1-30, wherein the starch-containing material is obtained from corn, cobs, wheat, barley, rye, milo, sago, cassava, manioc, tapioca, sorghum, rice or potatoes.

32. The process of any of claims 1-31, wherein the starch-containing material is obtained from cereals.

33. The process according to any of claims 1-32, wherein the temperature during step (a) is from 30°C to 75°C, preferably between 45 and 60°C.